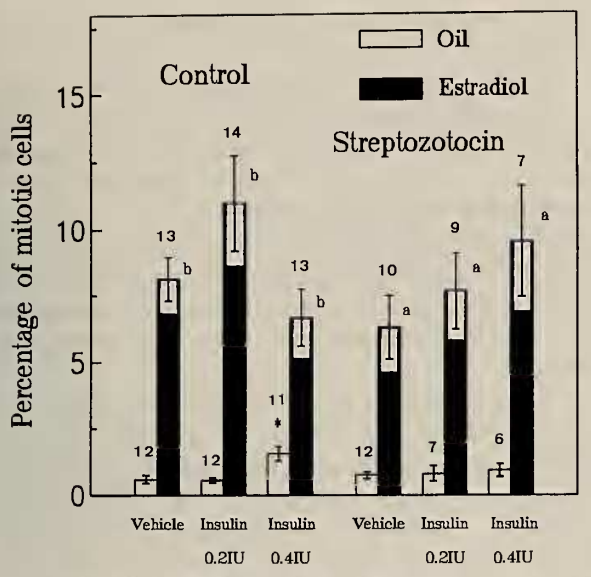

FIG. 3. Influence of estradiol-17β and insulin (0.2 IU, 0.4 IU) on the percentage of mitotic uterine luminal epithelial cells (mean ± SE of the mean) in control ovariectomized mice and streptozotocin-treated ovariectomized mice. The number above each column depicts the number of mice. *, $P<0.01$ *vs.* respective vehicle group. a, $P<0.01$; b, $P<0.001$ vs. respective oil group.

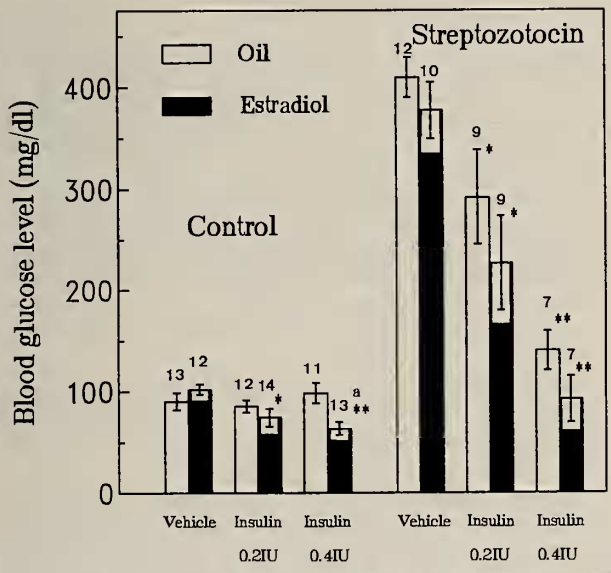

FIG. 4. Blood glucose levels in control ovariectomized mice and streptozotocin-treated ovariectomized mice receiving estradiol-17β or insulin. The number above each column depicts the number of mice. *, $P<0.05$; **, $P<0.01$ *vs.* respective vehicle group. a, $P<0.01$ *vs.* respective oil group.

treated mice (Fig. 3). Insulin administration in association with estrogen did not further increase the mitotic activity of luminal epithelial cells in both control and STZ-treated mice.

Blood glucose level

Normal blood glucose level was 90.1±8.4 mg/dl (n=13). Estrogen treatment did not change the blood glucose level, although in insulin-treated control mice estrogen decreased glucose level (Fig. 4). STZ treatment increased blood glucose levels (409.0±19.6 mg/dl, n=12, at 20 days after STZ injection). Insulin decreased blood glucose levels in a dose-dependent manner in STZ-treated mice.

DISCUSSION

The present study revealed that insulin stimulated the proliferation of pituitary cells. Growth promoting action of insulin on uterine epithelial cells had been already demonstrated in rats and mice [9, 15]. Rat pituitary tumor cells, GH_3 cells, require insulin for the optimal growth *in vitro* system [11]. However, as far as we know, stimulatory effect of insulin on the proliferation of normal pituitary cells has never been reported.

STZ administration induces diabetes in mice and rats, which is ascertained by serum hyperglycemia and significant loss of body weights. Insulin administration was able to decrease the elevated serum glucose levels in STZ-induced diabetic mice. Our preliminary study showed that STZ (100 mg/kg) injection significantly decreased serum insulin levels (control male mice, 51.1±8.9 μU/ml; STZ-treated males, 12.0±5.1 μU/ml).

In STZ-treated mice, estrogen failed to increase the mitotic activity of pituitary cells. Repeated injection of insulin recovered the reduced responsiveness of pituitary cells to estrogen in STZ-treated diabetic mice to the level observed in normal mice. Insulin (0.4 IU) increased the mitotic activity of luminal uterine epithelial cells in control mice. Ineffectiveness of insulin in STZ-treated mice on the proliferation of luminal cells may be accounted for by the reduced responsiveness of uterine epithelial cells to insulin in insulin-deficient mice. Insulin was not able to enhance further the estrogen-induced proliferation of uterine cells. These results indicate that insulin stimulates the cell proliferation in pituitary cells and uterine epithelial cells, but the pathway of signal transduction leading to cell division may be different.

Several studies described the proliferation of pituitary cells in rats [4, 22, 23, 28]. Growth hormone-secreting cells (somatotrophs) and PRL cells are the most actively proliferating cells in pituitary secretory cells. PRL cells were particularly analyzed in the present study, since PRL secretion is known to be regulated by insulin [13, 25]. STZ-treated diabetes decreased PRL secretion in rats [6, 12]. In PRL cells of such diabetic rats the decrease in number of secretory granules and the atrophy of cell organelles were electron microscopically observed [34]. The present study showed that insulin stimulated the proliferation of PRL cells. The enhanced proliferation of PRL cells by insulin may be correlated with the enhanced PRL secretion [29]. Further analysis is needed for the understanding of the secretion and proliferation-coupling of PRL cells.

IGF receptors as well as insulin receptors are localized in pituitary glands [10], and in uterine tissues [7]. Our preliminary *in vitro* study indicated that IGF-I was more potent (about 100-fold) in stimulating the proliferation of cultured

pituitary cells than insulin (Oomizu and Takahashi, unpublished observation). Therefore, insulin action may be mediated by insulin-like growth factor-I (IGF-I) receptors in pituitary glands.

Several studies indicate that estrogen action on the cell proliferation is indirect and mediated by autocrine or paracrine growth factors [24, 27, 32]. Transforming growth factor α and epidermal growth factor are candidates of growth factors in the pituitary gland and the uterus [3, 16, 19, 20, 31]. Insulin or IGF-I may be another candidate of estrogen-associated growth factors. IGF-I and IGF-I mRNA are detected in pituitary glands [1, 18, 21]. Estrogen increased levels of IGF-I mRNA, IGF binding and IGF binding proteins [17]. As mitogenic action of estrogen is well known, the increase in pituitary IGF-I level by estrogen treatment may be closely associated with the estrogen-induced proliferation of pituitary cells. Estrogen administration may stimulate the secretion of IGF-I from pituitary cells, and in turn IGF-I secreted may stimulate the proliferation of pituitary cells in an autocrine or paracrine fashion. It is highly probable that insulin administered in the present study is able to accelerate the pituitary cell proliferation by the stimulation of intrinsic pituitary IGF-I system. In the uterus IGF-I is also detected [18], and its synthesis is stimulated by estrogen [2, 8]. IGF-I receptors are detected in uterine cells [7]. These results strongly suggest the autocrine or paracrine control of IGF-I on the proliferation of uterine cells. Therefore, it is also highly probable that insulin acts on IGF-I receptors, resulting in the stimulation of uterine epithelial cells.

Reduced responsiveness to estrogen on prolactin secretion had already reported in STZ-treated rats [6, 30, 33], and this reduction is partly due to the alteration in pituitary estrogen receptor system [30, 33]. Most of pituitary secretory cells including PRL cells had estrogen receptors [14]. Ineffectiveness of estrogen on the proliferation of pituitary cells in STZ-treated mice may result from altered mechanism of estrogen receptors.

In the previous study using STZ-treated or alloxan-treated diabetic rats, the response of uterine epithelial cells to estrogen on the proliferation was significantly reduced, and this was restored by insulin treatment [15]. Their result does not agree with our result. They had used the lower dose of estradiol-17β (4 μg/100 g body weight) compared with the dose of estrogen used in the present study. As we clearly found the diminished response in pituitary cells with this estrogen dose, one possible reason for this discrepancy is that the uterine epithelial cells in rats may be more responsive to estrogen than the pituitary cells. Estrogen receptor kinetics and estrogen activity for protein synthesis were altered in the uteri of STZ-induced diabetic rats, and restored by insulin treatment [5]. Thus, STZ treatment in the rat more severely may affect the estrogenic mechanism in the uterus. We preliminarily found that insulin and IGF-I stimulated the proliferation of mouse uterine epithelial cells *in vitro* (Takahashi and Miyake, unpublished observation). Further study on insulin or IGF-I action on the mouse uterine cells is needed.

Chronic estrogen administration increases pituitary weights, which mainly results from the hypertrophy and hyperplasia of PRL cells [29]. Gala and Jaques [6] demonstrated that STZ-induced insulin deficiency retarded estrogen-induced pituitary growth in the rat. The retarded growth of pituitary glands in STZ-treated rats is thought to be partly due to the diminished mitotic activity of pituitary cells, since the lower mitotic activity of pituitary cells including PRL cells in STZ-treated mice was shown in the present study.

In conclusion, the present *in vivo* study clearly showed that insulin administration increased the cell proliferation of pituitary cells. In pituitary cells insulin was required for estrogen-induced cell proliferation. Molecular basis of insulin-estrogen interaction must be studied using the *in vitro* system.

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